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(6)

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A23L 1/302, 1/304, A61K 31/375, 31/525, 31/295</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/46090</b>
			<b>(43) International Publication Date:</b> 22 October 1998 (22.10.98)
<b>(21) International Application Number:</b> PCT/CA98/00342 <b>(22) International Filing Date:</b> 8 April 1998 (08.04.98)  <b>(30) Priority Data:</b> 2,202,686 15 April 1997 (15.04.97) CA 09/020,335 9 February 1998 (09.02.98) US  <b>(71) Applicant:</b> HOPITAL SAINTE-JUSTINE [CA/CA]; 3175 Côte Sainte-Catherine, Montréal, Québec H3T 1C5 (CA).  <b>(72) Inventors:</b> CHESSEX, Philippe; 17 Beverley Avenue, Ville Mont-Royal, Québec H3P 1K3 (CA). LAVOIE, Jean-Claude; 9610 des Mille-Iles, Laval, Québec H7A 4C3 (CA).  <b>(74) Agent:</b> COTE, France; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).			<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> ANTI-OXYDANT MULTIVITAMIN PREPARATION			
<b>(57) Abstract</b>  The present invention relates to a liquid multivitamin preparation with reduced amounts of peroxide. The present invention also relates to a liquid multivitamin preparation with reduced amounts of peroxide wherein the multivitamin preparation comprises iron bound to a polyose molecule or a combination of riboflavin and vitamin C, wherein both compounds are not in contact with each other. The present invention also concerns a method for administering liquid multivitamin with reduced amounts of peroxide. The present invention also concerns a dispensing means for administering liquid multivitamin with reduced amounts of peroxide and in order to protect the preparation from light.			
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## ANTI-OXYDANT MULTIVITAMIN PREPARATION

BACKGROUND OF THE INVENTION(a) Field of the Invention

5           The present invention relates to multivitamin preparation for enteral or parenteral administration. The present invention also relates to a method of administering multivitamin preparations.

(b) Description of the prior art

10           There has been a lot of research in the area of the oxidation of lipids found in foodstuffs mainly because of the negative economic impact caused by the unpleasant tastes and odors generated by the peroxidation of lipids. At the cellular level, the biological  
15 effects of peroxides are well documented. Their effect in the organism as a whole, where there is interactions between all anti-oxidant systems is not well documented. For newborn infants with an immature anti-oxidant capacity, the clinical effects of infused per-  
20 oxides is a cause of alarm. Parenteral nutrition is often required in the early stages of feeding immature very low birth weight infants. When compared to intra-venous glucose only, high levels of hydroperoxides have been detected in nutrient solutions and in complete  
25 preparations of intravenous nutrition. Hydroperoxides are reactive and can form free radicals in turn these oxygen species may disrupt cell membrane integrity and mediate tissue injury. They can also interfere with the production of vasoactive eicosanoids and stimulate  
30 neovascularization which is responsible for the retinopathy of prematurity. Because antioxidant systems of premature infants are immature, the potential risk of the peroxides to cause oxidant stress is high. There is a correlation between markers of free oxygen radi-  
35 cal-induced lipid peroxidation and outcome in immature

newborn infants. The role of those peroxides is questioned in the pathogenesis of bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of premature infants.

5 Under the conditions met during intra-venous nutrition of newborn infants, the multivitamin solution is the major source of hydroperoxides. It is well known that the generation of hydroperoxides is stimulated by phototherapy or even by day-light. There is  
10 therefore a need to decrease the oxidant load received by immature infants. Riboflavin and polysorbate are light sensitive molecules which could be implicated in the generation of peroxides. Most parenteral multivitamin solutions contain 5'-phosphate flavin mononucleotide (FMN) as a source of riboflavin, a vitamin implicated in a large number of metabolic pathways, including  
15 respiratory chain reaction. Polysorbates (PS) are detergents added in certain multivitamin solutions to dissolve liposoluble and hydrosoluble vitamins in the same medium.  
20

#### Oxidation of perfused lipids

The abundance and the chemical structure of fatty acids present in cell membranes renders them a preferred target for radical attack. A free radical  
25 (RL) is an atom of which the last electronic layer is occupied by a free electron. This particularity renders the free radical very reactive. In order to lower its energy level, the free radical will try to gain a further electron or loose the free electron. Double  
30 bonds of polyinsaturated fatty acids are a source of electrons for free radical. Free radicals will react in a non-specific way with different molecules. The free radicals will react by three different mechanisms. They can give their electron and become ionic, they can  
35 extract an electron from an other molecule thereby gen-

erating an other free radical or they can bind with the target molecule, this new molecule will then become a radical. The damages caused by the generation of free radicals are varied depending where free radicals are released. Damages include inactivation of proteins, modification of DNA and activation of other molecules. The list of pathologies related to oxidation is exhaustive and includes certain types of cancer, aging, rheumatoid arthritis, fibrosis, ischemia repression syndrome and artherosclerosis.

Peroxidation of lipids is a three-step chain reaction. The three steps are initiation, propagation, and termination. Initiation occurs when a free radical binds to a fatty acid(L) or extracts an electron from that fatty acid. The resulting molecule is then a radical(L•) which can react with molecular oxygen to form a hydroperoxide(LOO•). That radical will then attract an electron from an other fatty acid to yield a hydroxyperoxide(LOOH) and the reaction will continue with the radical generated in the process. The chain will stop when there is no more substrate or when an anti-oxidant molecule such as alpha-tocopherol provides the missing electron. Depending on the type of fatty acid and the site of the radical attack, there is formation of different molecules. Since the dissociation energy of a C-H bond of a bi-allyl structure is weaker then that of a mono-allyl structure (75 Vs 88 Kcal/mol), polyunsaturated fatty acids will more likely loose their electron to become a radical with which oxygen will bind to form a hydroperoxide. During the addition, a conjugated diene is produced which can be detected by spectroscopy at 230-235 nm. The LO-OH bond of the hydroxyperoxide has a dissociation energy of about 44 Kcal/mol. An electron donor such as  $\text{Fe}^{2+}$  or  $\text{Cu}^+$  will be sufficient to generate an alkoxyl radical.

That alkoxyl radical can attract an electron from a fatty acid and maintain the lipid peroxidation. However, a break in a C-C bond will generate an aldehyde and an alkane. If a molecule of O<sub>2</sub> is added to the double bond near the methyl moiety of the fatty acid, pentane is formed from n-6 fatty acids, and ethane is derived from n-3 fatty acids. These gases are measured in breath as markers of peroxidation of endogenous and/or exogenous lipids. The most commonly measured aldehydes are hexanal, 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA).

Quantification of hydroperoxides, alkanes, aldehydes, conjugated dienes, as well as molecules produced by the action of free radicals on proteins or DNA are used as markers of oxidative stress. However, the presence or absence of such markers are not absolute proof that oxidation did or did not take place. These chemical compounds take part in the general metabolism, therefore their generation is not solely due to lipid peroxidation. For instance, MDA, hexanal and HNE can all be catabolized by either aldehyde dehydrogenase or glutathione-S-transferase. Sources of MDA are diverse, for instance thromboxane synthetase activity will produce one molecule of MDA for each thromboxane A<sub>2</sub>. Alkanes produced by fermentation in the gastrointestinal tract can contaminate alkanes measured in breath.

The presence of markers of lipid peroxidation suggests that the antioxidant capacity of the cell was at one point overwhelmed. Furthermore, cell damage can appear before or at the same time as the markers of peroxidation. Certain markers of lipid peroxidation are toxic. Aldehydes can form carbonyl moieties on macromolecules, or react with the lysine moieties of proteins. Such reactions can inactivate glucose-6-phosphate dehydrogenase or activate collagen formation.

Furthermore, peroxides are potentially highly oxidant since providing them with one electron will produce a reactive oxygen radical. Hence peroxides can influence, alone or as free radicals, the formation of vasoactive molecules such as prostaglandins or thromboxanes.

Because of their high polyunsaturated fatty acid concentration, lipid emulsions are a target for peroxidation. Hence, emulsions containing a mix of medium and long chain fatty acids can be advantageous as they decrease the amount of unsaturation. Other nutrients also generate peroxides such as certain amino acids, vitamins, trace metals. Because of the immaturity of the antioxidant system of premature infants, infused peroxides are potentially cytotoxic and enhance mortality and morbidity. Lavoie et al. (*Pediatrics*, 1997, 99 (3):1-5) discuss the role of a multivitamin preparation on the actual peroxide load received by patients. This publication describes that even when protected from light, the multivitamin preparation infused produced a 10 fold increase in peroxide.

In adult patients, parenteral concentrations of multivitamins are lower than those required for newborn infants, and the rate of infusion is faster. Therefore, one can speculate that the infused peroxide load is lower in adult patients. However, in compromised subjects the role of infused peroxides on the pathogenesis of morbid complications needs to be further investigated. Indeed, in patients with sepsis and adult respiratory distress syndrome, urinary peroxides concentrations were consistently higher in patients who did not survived.

Multivitamin solutions contain several antioxidants such as ascorbate (vitamin C), tocopherol (vitamin E) and vitamin A. They have free radical

scavenging properties but no antiperoxide activity. To date, no known source of enteral or parenteral multivitamin solutions protects against the generation of peroxides.

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#### SUMMARY OF THE INVENTION

It is an aim of the present invention to provide a liquid multivitamin preparation with reduced amounts of peroxide.

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It is an aim of the present invention to provide a liquid multivitamin preparation for enteral or parenteral administration with reduced amounts of peroxide.

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It is an aim of the present invention to provide a liquid multivitamin preparation comprising riboflavin and vitamin C for enteral or parenteral administration with reduced amounts of peroxide wherein the riboflavin is not in contact with the vitamin C.

20

It is an aim of the present invention to provide a liquid multivitamin preparation with reduced amounts of peroxide wherein the multivitamin preparation is solubilized in the presence of iron bound to a polyose molecule.

25

It is an aim of the present invention to provide a liquid multivitamin preparation for enteral or parenteral administration with reduced amounts of peroxide wherein the multivitamin preparation is solubilized in the presence of iron bound to a polyose molecule.

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It is also an aim of the present invention to provide a liquid multivitamin preparation comprising riboflavin and vitamin C for enteral or parenteral administration comprising reduced amounts of peroxide wherein the multivitamin preparation comprises two phases until the time of enteral or parenteral administration,

35

a riboflavin phase separated from a vitamin C



phase up to a molar ratio about 1/10,000 between concentrations of riboflavin and vitamin C.

It is also an aim of the present invention to provide a liquid multivitamin preparations for enteral  
5 or parenteral administration comprising reduced amounts of peroxide wherein the multivitamin preparation does not comprise riboflavin, which is administrated separately.

It is also an aim of the present invention to  
10 provide a method of administering multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the step of protecting the multivitamin preparation from light.

It is also an aim of the present invention to  
15 provide a substantially opaque dispensing means for administering multivitamin preparation in order to protect the multivitamin preparation from light.

In accordance with the present invention there  
20 is provided a liquid multivitamin preparation comprising riboflavin and vitamin C for enteral or parenteral administration and a reduced amount of peroxide, wherein the riboflavin is not in contact with the vitamin C.

In accordance with the present invention there  
25 is also provided a liquid multivitamin preparation for parenteral or enteral administration comprising reduced amounts of peroxide, wherein the multivitamin preparation is solubilized in the presence of iron bound to a  
30 polyose molecule.

The iron bound polyose molecule may be selected from the group consisting of Iron Saccharide, Iron Dextran and Iron Sorbitol. The preferred iron bound polyose molecule is Iron Dextran.

In accordance with the present invention there is also provided a liquid multivitamin preparation comprising riboflavin and vitamin C for enteral or parenteral administration and a reduced amount of peroxide, wherein the multivitamin preparation is in two phases until the time of enteral or parenteral administration, a riboflavin phase separated from a vitamin C phase up to a molar ratio of about 1/10,000 between concentrations of riboflavin and vitamin C.

10 In accordance with the present invention there is also provided a method of administering a liquid multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide, wherein the method comprises the step of protecting the preparation  
15 from light.

The light may have a wavelength of about 200nm to about 800nm, of about 300nm to about 600nm, or more particularly of about 325nm to about 550nm.

The step of protecting the preparation from  
20 light may be effected by means of a substantially opaque dispensing means for administering the multivitamin preparation, or by means of a colored dispensing means for administering the multivitamin preparation. The dispensing means may be colored by at least one  
25 color or a combination thereof selected from the group consisting of black, gray, brown, orange, yellow, green, blue, and red, more preferably by a color or a combination thereof selected from the group consisting of black, brown, orange and yellow.

30 In accordance with the present invention there is also provided a colored container or a substantially opaque container for administering a liquid multivitamin preparation in order to protect the multivitamin preparation from light.

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**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 demonstrates the influence of lipid emulsion and day light on peroxide concentration of par-  
enteral nutrient solution freshly prepared and compris-  
5 ing multivitamins;

Fig. 2 demonstrates the effect of two different  
sources of iron bound to a polyose molecule namely Iron  
Dextran (FeD) and Iron sorbitol (FeS) on the generation  
of peroxides in parenteral nutrient solution;

10 Fig. 3 demonstrates the effect of polysorbate  
detergents 80(1%) and 20(0.02%) on the generation of  
peroxides in a control solution (glucose 5%g + NaCl  
0.45g% + heparin 1U/ml) and in a multivitamin solution  
(VIT);

15 Fig. 4 demonstrates the role of riboflavin and  
ascorbic acid (vitamin C) with the generation of perox-  
ide in the multivitamin solution and in the control  
solution (glucose 5%g + NaCl 0.45g% + heparin 1U/ml);

20 Fig. 5 demonstrates the level of peroxides in  
the TPN solution (all in one) exposed 3 hours to day-  
light (control) in presence of increasing concentra-  
tions of 4 sources of iron;

Fig. 6 demonstrates the effect of increasing  
concentrations of 4 sources of iron on 500µM H<sub>2</sub>O<sub>2</sub> (A),  
25 TBH: tert-butyl hydroperoxide (B) and cumene hydroper-  
oxide (cumene) (C);

Fig. 7 demonstrates the free oxygen radicals  
produced by the Fenton reaction are detected by oxida-  
tion of 3 µM scopoletin;

30 Fig. 8 demonstrates the effect of iron on the  
generation of peroxides in the TPN solution (all in  
one) exposed 0 to 3 hours to day-light;

Fig. 9 demonstrates the effect of increasing  
concentrations of 3 sources of iron on the availability  
35 of the thiol function of cysteine;

Fig. 10 represents the experiment design of Example 3;

Fig. 11 represents the peroxide concentrations in fat-free TPN delivered with the 5 different intravenous settings, exposed to ambient light;

Fig. 12 represents the thiol functions in fat-free TPN delivered with the 5 different intravenous settings, exposed to ambient light;

Fig. 13 represents the peroxide concentrations in fat-free TPN delivered with 5 intravenous settings exposed to phototherapy;

Fig. 14 represents peroxide concentrations in a all-in-one TPN solution (with lipid emulsion) delivered with the 5 different intravenous settings, exposed to ambient light; and

Fig. 15 represents the spectra of absorbance of tested amber tubing (yellow, orange) and of 5'-phosphate flavin mononucleotide.

## 20 DETAILED DESCRIPTION OF THE INVENTION

Specifically, the present invention concerns multivitamin solutions with a reduced peroxide content.

More specifically, the present invention concerns parenteral and enteral multivitamin solutions with a reduced peroxide content. The present invention also concerns parenteral and enteral multivitamin solutions with a reduced peroxide content.

The generation of peroxides in multivitamin solutions is caused by the interaction between riboflavin and ascorbate in presence of light. Because of the immaturity of the antioxidant capacity of premature infants, infused peroxides are potentially cytotoxic and might increase morbidity and mortality

By the term "polyose molecule" is meant a polysaccharide or an analogue or derivative thereof. The

term "polysaccharide" include naturally occurring polysaccharides and synthetic polysaccharides. Polysaccharides can be easily derivatized at functional groups by methods well known in the art. For example, modifications include but are not limited to adding an amino acid, a peptide or a polypeptide to the polysaccharide moiety.

A suitable polyose molecule is capable of binding to an iron atom to form a complex. The polyose molecule has to be selected such that when it is bound with the iron atom the complex formed does not promote formation of free oxygen radical when it is added to a multivitamin preparation.

The term "multivitamin preparation", is meant to be interpreted broadly and include suitable preparation and solution used in intravenous nutrition and oral vitamin supplement.

By the term "reduced amounts of peroxide" is meant a reduction of the amounts of peroxide in a test preparation when compared to a control preparation.

By the term "peroxides" is meant harmful peroxides generated in multivitamin preparation and nutrient solution.

Preferably the iron bound polyose molecule is selected from the group consisting of Iron Saccharide, Iron Dextran and Iron Sorbitol.

More preferably the iron bound polyose molecule is Iron Dextran or Iron sorbitol.

Most preferably the iron bound polyose molecule is Iron Dextran.

In a preferred embodiment, there is provided a method of administering a liquid multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the

step of protecting the preparation from light having a wavelength of about 200nm to about 800nm.

In a preferred embodiment, a method is provided for administering a liquid multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the step of protecting the preparation from light having a wavelength of about 300nm to about 740nm.

In a preferred embodiment, a method is provided for administering multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the step of protecting the preparation from light having a wavelength of about 300nm to about 600nm.

In a most preferred embodiment, a method is provided for administering multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the step of protecting the preparation from light having a wavelength of about 300nm to about 550nm.

In a most preferred embodiment, a method is provided for administering multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the step of protecting the preparation from light having a wavelength of about 325nm to about 550nm.

In a preferred embodiment, a method is provided for administering a liquid multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the step of protecting the preparation from light having a wavelength of about 500nm to about 600nm.

In a preferred embodiment, a method is provided for administering a liquid multivitamin preparation comprising riboflavin and vitamin C with reduced

amounts of peroxide wherein the method comprises the step of protecting the preparation from light by means of a substantially opaque dispensing means for administering the multivitamin preparation.

5           In a preferred embodiment, a method is provided for administering a liquid multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide wherein the method comprises the step of protecting the preparation from light by means  
10 of a colored dispensing means for administering the multivitamin preparation.

          In a further preferred embodiment the dispensing means is colored by one or more color or a combination thereof selected from the group consisting of black,  
15 gray, brown, orange, yellow, green, blue, and red.

          More preferably, the color is selected from the group consisting of black, brown, orange and yellow.

          Most preferably, the color is orange or yellow.

          In a most preferred embodiment, the color is  
20 yellow.

          The multivitamin preparation can be administered either parenterally or enterally. Depending of the case, the physician can select the appropriate route of administration.

25           The terms "parenteral or parenterally" refer to an administration that occurs elsewhere in the body than in the alimentary canal.

          The terms "enteral and enterally" refer to an administration that occurs in the alimentary canal and  
30 intestine.

          By the term "dispensing means" is meant a suitable device or apparatus for administering the multivitamin preparation to the patient. Dispensing means include but are not limited to medical bag for intravenous  
35 feeding, bottle for intravenous feeding, and con-

tainer for storing multivitamin solution (including container used for commercial sales of multivitamin solutions). The term is also meant to include tubing and other parts which may be necessary for administering the multivitamin preparation.

Substantially opaque or colored medical bags, tubing, and bottle can be prepared by methods well known in the art. The medical bags, tubing and bottle will have to be made of materials which do not react with the solution to be placed therein. Colored plastic can be used to manufacture the medical bags, tubing or bottle in order to achieve the aim of the present invention. It is also possible to cover the dispensing means with suitable substantially opaque or colored material in order to achieve the aim of the present invention. It is preferable however, that the multivitamin solution or nutrient solution be protected from light as soon as it is prepared since the generation of peroxides is a photoreaction which occurs very rapidly.

Suitable medical bags include Clintec™ Viaflex™ container manufactured by Baxter.

Suitable tubing include light protected tubing set by Baxter s.p.a. under the name Miraflo™ and Mixieva™ LPA, Miramed product line, Mirandola, Italy. Substantially opaque or colored medical bags can be prepared in the same fashion as the Miraflo™ tubing.

#### **Factors involved in the generation of peroxides in parenteral nutritive solutions**

Using an HPLC technique, Helbock et al. showed that different batches of the lipid emulsion Lyposin 20%™ (Abbott, USA), made with soybean and safflower oils, were contaminated with 290 mM peroxides, while the Lyposin 10% preparation contained half that amount. Using a colorimetric method (Jiang Z.Y. et al., *Lipids* 1991, 26:853-856) it was confirmed that Intralipid™ 10%



(Kabi-Vitrum, Sweden) made of soybean oil contained a similar concentration of peroxides (134 mM) as the 10% emulsion with the mixture of soybean and safflower oils (Lavoie J.C. et al., *Pediatrics*, 1997, 99 (3):1-5).

5       The colorimetric method was used to measure hydroperoxides is based on the oxidation of xylenol orange (Jiang Z.Y. et al., *Lipids* 1991; 26:853-856). In brief, at low pH,  $\text{Fe}^{2+}$  is oxidized in presence of hydroperoxides. The formed  $\text{Fe}^{3+}$  reacts with a chromo-  
10       phore (xylenol orange) which absorbs at 560 nm, linearly with a wide variety of peroxides such as  $\text{H}_2\text{O}_2$ , tert-butylhydroperoxide, cumene hydroperoxide (Lavoie J.C. et al., *Biochem. Pharmacol.*, 1994, 47:871-876).

      Because of their high polyunsaturated fatty acid  
15       content, lipid emulsions are thought of as an important source of peroxides. Hence, the advantage of mixed emulsions containing medium and long chain fatty acids, which decrease the amount of unsaturation. Other nutrients are known to generate peroxides, such as cer-  
20       tain amino acids, vitamins, trace metals. Bhatia and his colleges documented that in the presence of multivitamins certain amino acids (tryptophan, tyrosine, methionine, cysteine and phenylalanine) were photooxidized by blue light. The production of  $\text{H}_2\text{O}_2$  was pro-  
25       portional to the concentration of riboflavin. Furthermore, in presence of free iron,  $\text{H}_2\text{O}_2$  can generate, via the Fenton reaction ( $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^\bullet + \text{OH}^-$ ), highly reactive radical oxygen species. This under-  
30       lines the importance of adding only bound iron to parenteral solutions. Depending on their final concentrations, bisulfites added as stabilizing agents to a variety of intravenous preparations have antiperoxide as well as prooxidant properties (Lavoie J.C. et al., *Biochem. Pharmacol.*, 1994, 47:871-876). Metabisulfite  
35       has antiperoxide properties at the concentration (1.5

mM) found in the amino acid solution Travasol™ from Baxter, whereas at higher concentrations it can become prooxidant due to the presumed formation of a sulfite radical. The multivitamin preparation MVI Pediatric™  
5 from Rhône-Poulenc-Rohrer contains several antioxidants such as ascorbate, tocopherol, vitamin A, mannitol, butylhydroxytoluene. But it also contains detergents to improve the solubility of the liposoluble elements. The multivitamin preparation MVI Pediatric™ which is  
10 widely used in neonatology throughout North America contains polysorbates 20 & 80 at concentrations of 0.02 and 1% (v/v), respectively. These detergents are susceptible to peroxidation following photooxidation.

Researchers have mostly limited the investigation of the level of peroxidation to individual components of parenteral solutions. Recently, the concentration of peroxides found in complete solutions of TPN was measured the *in vitro* and *in vivo* in order to verify if the addition of a multivitamin preparation might  
20 protect the nutrients from oxidation (Lavoie J.C. et al., *Pediatrics*, 1997, 99 (3):1-5). It was found that, in the presence of light, the 1% (v/v) multivitamin preparation MVI was the major source of peroxides in the complete TPN solution. The admixture of a lipid  
25 emulsion had only a 10% additive effect over the initial peroxide concentration (Fig. 1). The concentrations of nutrients used was clinically relevant. The data from Fig. 1 represent the mean  $\pm$  SEM,  $n=3$ ; variability is not shown when its value is smaller than  
30 that of the symbols. The level of peroxides increased ( $p<0.001$ ) over time, and light exposure had a positive significant effect on the generation of peroxides ( $p<0.001$ ). The lipid emulsion had only an additive effect (Lavoie J.C. et al., *Pediatrics*, 1997,  
35 99(3):1-5).

This observation leads to the conclusion that *in vitro* this multivitamin preparation which is known for its antiradical properties, does not protect the TPN solution against peroxidation.

5           It is important to note that in presence of iron bound to carbohydrate with an elevated molecular weight (polyose), the generation of peroxides does not take place. Fig. 2 documents that clinically relevant concentrations of iron bound to a polyose protects a TPN  
10 solution against the generation of peroxides, even in the presence of light.

          As the multivitamin preparation MVI contains polysorbate, known to be photooxidizable, these experiments were repeated with a further polysorbate-free  
15 multivitamin preparation (Cernévit™ "VIT" Clintec Nutrition, Sèvre, France) which allowed the comparison of the levels of peroxides in the presence and the absence of added polysorbates 80 (1%) and 20 (0.02%). The multivitamin preparation VIT is commercially avail-  
20 able in Europe for intravenous use in adult patients. It contains higher concentrations of most vitamins. The experiments were performed *in vitro* at room temperature and day light with 1% VIT in 5% glucose + 0.45% NaCl + 1U/ml heparin to avoid contamination by  
25 peroxides generated by amino acids and lipids. Fig. 3 shows that VIT generates levels of peroxides comparable to MVI (Lavoie J.C. et al., *Pediatrics*, 1997, 99 (3):1-5). Furthermore, polysorbate alone has no effect, and its admixture to VIT modifies only the rate of peroxide  
30 generation but not the level. The data from Fig. 3 represent the mean  $\pm$  SEM, n=3; variability is not shown when its value is smaller than that of the symbols. The level of peroxides in VIT increases over time (p<0.001). Adding the detergents to VIT at the same

concentration as found in MVI does not modify significantly the level of peroxides.

When left in darkness the level of peroxides does not rise. Hence polysorbates are not the major  
5 source of peroxides.

Since this photoinduced generation of peroxides in the multivitamin preparations is independent from the detergents we evaluated the role of further components of the multivitamin preparation reported to be  
10 oxidizable, namely riboflavin and ascorbate. Indeed, ascorbate is an antioxidant known to become prooxidant at high concentration. Fig. 4 shows that separately riboflavin (22mM) and ascorbate (1.42 mM) did not generate peroxides. The data from Fig. 4 represent the  
15 mean  $\pm$  SEM, n=3; variability is not shown when its value is smaller than that of the symbols. However, the association of riboflavin and ascorbate induced an increase in peroxides over time up to concentrations similar to those found with the commercialized multivitamin preparations.  
20

The association of these two vitamins at concentrations found in 1% VIT produced an increase in peroxides over time which was similar to that found with VIT (Fig. 3) or MVI (Lavoie J.C. et al., *Pediatrics*, 1997,  
25 99 (3):1-5). The generation of peroxides is completely inhibited by catalase (50U/ml) suggesting that we are dealing with H<sub>2</sub>O<sub>2</sub>. This observation is corroborated by studies documenting by resonance spectroscopy that there is indeed an interaction between riboflavin and  
30 ascorbate during photooxidation. These results confirm that multivitamins represent the major source of peroxides infused to premature infants. These data suggests that infusing separately the hydrosoluble vitamins from the liposoluble vitamins as done by several teams will

not modify the generation of peroxides caused by the association of riboflavin and ascorbate.

At the bedside, the concentration of peroxides received by parenterally fed premature infants was measured. Light exposure had a dramatic effect on the generation of peroxides (Lavoie J.C. et al., *Pediatrics*, 1997, 99 (3):1-5). During the 4 hrs transit time from the TPN bag to the site of infusion, the level of peroxides increased by 50% (from 141 to 215 mM). But, when protecting the intravenous tubing from light, the level of infused peroxides remained at the same level as in the TPN bag. This was observed in the absence of lipids. Therefore, peroxidation is not limited to lipid emulsions. It is questionable whether these peroxides will produce detectable biological effects in the whole organism where all the antioxidants interplay.

#### Biological and clinical effects of infused peroxides

The level of peroxides infused with a solution of complete TPN (200-400 mM) corresponds with the amount of H<sub>2</sub>O<sub>2</sub> released by 10'000 granulocytes/ml/24 h during phagocytosis. This comparison underlines that the load of infused peroxides is of biological significance and that the body should therefore be equipped to contain such an oxidative load. But a weak peroxidase activity as found in the lung and liver of newborn and especially of premature infants suggests that infused peroxides are potentially cytotoxic.

There is a wide body of evidence that at the cellular level lipids and peroxides may have deleterious effects. Adding 10% Intralipid™ to erythrocytes will cause hemolysis. This reaction is catalyzed by free iron and inhibited by antioxidants such as ascorbate and vitamin E. This observation suggest that the

oxidant load associated with the lipid emulsion is responsible for the cytotoxicity. This is supported by the fact that tert-butylhydroperoxides stimulates proteolysis of erythrocytes at concentrations lower than  
5 what we found *in vitro* in TPN solutions. The addition of this same peroxide to red cells produces an increase in a marker of peroxidation (malondialdehyde) at concentrations proportional to membrane arachidonic acid. In turn, this points out the effect of oxidants on mem-  
10 brane fatty acids. Similarly, the addition of long chain triglycerides to liver, lung or intestinal homogenates results in an increase in malondialdehyde. On the other hand, it is worth noting that the cellular effects of peroxides are not all deleterious. As men-  
15 tioned before, they can interact with DNA and thereby stimulate the synthesis of antioxidant enzymes. Along the same line, Lee Frank has hypothesized that, at the end of gestation, an increase in free radical formation in the mitochondrion was the stimulus responsible for  
20 triggering in the lungs the expression of the genes coding for the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase.

Hydroperoxides contaminating solutions of parenteral nutrition modify the production of certain  
25 vasoactive eicosanoids leading to a vasoconstriction which results in a decreased pulmonary perfusion and a deterioration in gas exchanges. There is a wide body of evidence suggesting an effect of oxidants on eicosanoids. Indeed, in an umbilical vein model we confirmed  
30 that a free radical generating system contributes to decrease the production of prostacyclin (PGI<sub>2</sub>), while peroxides stimulate globally the production of prostaglandins. Lipids have a combined effect resulting in vasoconstriction due to a decrease in the production of  
35 the vasodilating PGI<sub>2</sub> and a stimulation of the vasocon-

stricting eicosanoid thromboxane A<sub>2</sub> (TxA<sub>2</sub>). A similar stimulation of TxA<sub>2</sub> was observed in the lungs of rabbits perfused with reactive oxygen species.

In the course of studies on the effect of energy  
5 substrates on respiratory gas exchanges in premature infants, we observed that a lipid-rich regimen induced a deterioration compared to an isocaloric glucose-rich regimen. Following normal physiological ventilatory  
10 adaptations to an elevated glucose load, there was an increased CO<sub>2</sub> production and pCO<sub>2</sub> remained unchanged. But, there was a statistical difference in pO<sub>2</sub> between both isocaloric regimens, with the lipid regimen exhibiting the lower value. Three different publications  
15 report a similar observation under comparable experimental conditions. Although pO<sub>2</sub> values were within a normal range, the fact that different groups report the same observation in different populations suggests a biological effect. These results may be explained by an increase in minute ventilation with the glucose-rich  
20 regimen. Alternatively, based on our *in situ* findings of a vasopressor effect of the lipid emulsion in the umbilical vein model we believe that the drop in pO<sub>2</sub> during the lipid-rich regimen is related to a vasoconstriction mediated by prostaglandins. However, if the  
25 production of prostaglandins was induced mainly by the peroxides contaminating the TPN solutions, and if the multivitamin preparation was the major source of peroxides, one would not expect to find any difference between the isocaloric regimens differing by 4 g/kg/d  
30 of glucose and 1.2 g/kg/d of lipids. Hence, it is possible that the effects on the respiratory gas exchange is not mediated solely by the infused peroxides. As suggested by others, the prostaglandin precursors infused with the lipid emulsion might also stimulate  
35 directly eicosanoid synthesis.

Following published reports of an increased morbidity represented by bronchopulmonary dysplasia in preterm infants receiving a lipid emulsion, it is questionable whether this association is related to infused peroxides. In an animal model, pentane measured in the breath of newborn pups was proportional to the dose of infused lipids. Therefore to investigate the clinical response induced by infused peroxides, the effect of this oxidant has to be separated from that of the lipid emulsion. This is what Pitkänen et al. did when studying premature infants less than five days of age who were receiving a fat-free nutrient intake and who were all ventilated. They found that the infants with elevated indices of peroxidation represented by exhaled ethane and pentane had a greater ( $p < 0.05$ ) probability of death or of developing bronchopulmonary dysplasia. This suggests that peroxides are of clinical significance in premature infants.

To reduce the deleterious effects associated with oxidants, it would be relevant to stimulate the antioxidant capacity of newborn infants at risk. Furthermore, we need to find ways to reduce the generation of peroxides induced by our therapeutic approaches and find preparations with antiradical as well as antiperoxide properties.

Other characteristics and advantages of the present invention will appear from the following examples. The following examples are intended to document the invention, without limiting its scope.

30

#### Example I

##### **Preparation of a multivitamin solution**

A multivitamin preparation suitable to be administered parenterally with reduced amount of infused peroxides can be prepared as generally described below.

35



Riboflavin is solubilized with liposoluble vitamins while vitamin C (ascorbate acid) is solubilized with hydrosoluble vitamins. The liposoluble vitamins being separated from the hydrosoluble vitamins and administered separately. Since the riboflavin is not in contact with the vitamin C there is no generation of peroxides.

#### Example II

##### 10    **Prevention of generation of peroxides in multivitamin solution with Iron Dextran**

Iron dextran (Infufer™) and iron sorbitol (Jectofer™) at 50 mg iron/ml were obtained from Sabex International (Montreal, Que, Canada). FeCl<sub>2</sub>, FeSO<sub>4</sub>, FeCl<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, tert-butyl hydroperoxide (TBH), cumene hydroperoxide, scopoletin, HCl-cysteine, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), xylenol orange [o-cresolsulfonphthalein-3',3''-bis(methyliminodiacetic acid sodium salt)], 2,6-di-tert-butyl-4-methyl-phenol (BHT) and sorbitol were purchased from Aldrich Chemical Corp (Milwaukee, Wis, USA). Dextran 5 kDa was provided by Sabex, as the same source of dextran used for FeD, while dextran 10 kDa was purchased from Pharmacia Inc (Mississauga, Ont, Canada). TRIS and EDTA-Na<sub>2</sub> were obtained from Boehringer-Mannheim (Laval, Que, Canada). The 10% Intralipid™ emulsion was purchased from Pharmacia. The 10% amino acid solution Travasol™ in glass containing 300 mg/L of m-bisulfite (BlendC) was obtained from the Clintec Nutrition Company (Mississauga, Ont, Canada). The multivitamin preparation MVI-Paediatic™ (5 ml provided 2300 IU vitamin A, 400 IU vitamin D, 7 IU vitamin E, 200 µg vitamin K, 80 µg vitamin C, 1.2 mg thiamine, 1.4 mg riboflavin, 1.0 mg pyridoxine, 17 mg niacin amide, 5 mg pantothenate, 140 µg folic acid, 1 µg vitamin B<sub>12</sub> and 20 µg

biotin) was purchased from Rhône Poulenc Rorer (Montreal, Que, Canada). The trace element preparation "Micro + 6 Pediatric™" (1 ml provided 3 mg zinc, 0.4 mg copper, 0.1 mg manganese, 4 µg chromium, 20 µg selenium, 60 µg iodine) was obtained from Sabex International (Montreal, Que, Canada).

The levels of hydroperoxides were measured in 4 protocols:

- in the TPN solution, after a 3-hour incubation at day light and room temperature (control) in presence of different sources of iron. FeCl<sub>2</sub>, FeSO<sub>4</sub>, FeD (iron bound to dextran) and FeS (iron bound to sorbitol) were used at concentrations ranging from 0 to 0.8 mM;
- in non-TPN solutions containing 500 µM H<sub>2</sub>O<sub>2</sub>, TBH and cumene hydroperoxide, after a 0.5-hour incubation at day light and room temperature in presence of the 4 sources of iron, at concentrations ranging from 0 to 0.8 mM;
- in the TPN solution after a 0, 1, 2 and 3-hour incubation at day light and room temperature in presence of 100 µM FeD, FeS, and equivalent amounts of dextran 5 kDa & 10 kDa and sorbitol; and
- in the lipid-free TPN solution after a 0 to 3-hour incubation at day light and room temperature in presence of 100 µM FeD.

The TPN solution was designed to provide clinically relevant concentrations of individual nutrients delivered to newborn infants: 2.5% (w/v) amino acids + 10% (w/v) dextrose + standard electrolytes and trace elements + 1% (v/v) multivitamin preparation MVI Ped + 5.5% (v/v) lipid emulsion. The solution was prepared in water.

To quantify hydroperoxides, the ferrous oxidation/xylenol orange technique was used as described previously (Jiang ZY et al., *Lipids*, 1991, 26:853-856);

at low pH,  $\text{Fe}^{2+}$  is oxidized in the presence of hydroperoxides; the formed  $\text{Fe}^{3+}$  reacts with xylenol orange to produce a chromophore which absorbs at 560 nm linearly with the concentration of a wide range of hydroperoxides (Lavoie JC et al., *Biochem. Pharmacol.*, 1994, 47:871-876). In the present study, 22.5 mM  $\text{H}_2\text{SO}_4$ , 90  $\mu\text{M}$  xylenol orange, 225  $\mu\text{M}$   $\text{FeCl}_2$  and 3.6 mM BHT were added to 100  $\mu\text{L}$  of sample, for a total volume of 1 ml. The absorbance was read (Beckman spectrophotometer DU-6) after 30 min. incubation at room temperature. Intra- and inter-assay coefficients of variation were 4 and 5%, respectively. Hydroperoxides were expressed in  $\mu\text{M}$  equivalents of TBH ( $\mu\text{M}$  equ TBH).

Free oxygen radicals were detected using the fluorescent property of Scopoletin, which is modified in relation to its level of oxidation (Lavoie JC et al., *Biochem. Pharmacol.*, 1994, 47:871-876). Briefly, in TRIS buffer (50mM TRIS, 0.1 mM EDTA, pH 7.7), 100  $\mu\text{M}$  iron ( $\text{FeCl}_2$  or  $\text{FeSO}_4$ ) were added to 3  $\mu\text{M}$  reduced scopoletin, in presence of  $\text{H}_2\text{O}_2$  (0, 50 and 125  $\mu\text{M}$ ). After activation at 350 nm, a drop in fluorescence at 460 nm, was associated with an oxidation of scopoletin by reactive oxygen species. Separately, iron or  $\text{H}_2\text{O}_2$  had no effect on oxidation of scopoletin.

The proportion of iron freed by FeD and FeS was quantified by taking advantage of the iron binding property of the thiol function. The masking effect of FeD and FeS on the thiol function of cysteine, using  $\text{FeCl}_3$  as a positive control were compared. Iron (0 to 0.4 mM) was added to a solution of TRIS buffer containing 100 $\mu\text{M}$  HCl-cysteine. After incubation for 30 min. at room temperature, 0.6 mM of DTNB was added. The absorbance was read at 412 nm (Ellman GL, *Arch. Biochem. Biophys.*, 1959, 82:70-77); corresponding cysteine-free blanks were subtracted.

Fig. 5 shows the dose-response relationships between increasing concentrations of 4 different sources of iron on the level of peroxides in a TPN solution. At 100  $\mu\text{M}$  iron, peroxide concentrations were 75% lower than the control (TPN solution). At iron concentrations  $>200 \mu\text{M}$ , FeD appeared to have a separate effect by reaching a steady state. Since it is well known that the level of peroxides in TPN increases with time, the low iron-induced level of peroxide could be caused either by a consumption in a Fenton-like reaction or a protection against the generation of peroxides.

In order to verify if the interaction of iron with peroxides resulted from the consumption or the inhibition of peroxide generation, the direct effect of iron on different sources of peroxides were measured (Fig. 6). As opposed to bound-iron,  $\text{FeCl}_2$  and  $\text{FeSO}_4$  produced a marked drop in peroxides. The consumption of peroxides by both these sources of free iron is confirmed by the concomitant increase in free oxygen radical formation shown in Fig. 7. The absence of a consumption of peroxides by bound iron sources (Fig. 2) led us to verify if FeD and FeS were involved in the generation of peroxides. The results from Fig. 8 show that the spontaneous light-induced generation of peroxides over time was contained by both sources of bound iron, and not associated with an initial consumption. In the lipid-free TPN solution, FeD had a similar protective effect on the light induced generation of peroxides over time. Indeed, the lipid-free TPN solution was associated with a significant increase in peroxides over 3 hours of exposure to light (from  $52.2 \pm 8.3 \text{ mM eq. TBH}$  at 0 h to  $104.0 \pm 11.1 \text{ mM eq. TBH}$  at 3h), which was abolished with FeD ( $42.6 \pm 2.6 \text{ mM eq. TBH}$  at 3 h). The role of dextran 5 kDa, 10kDa and sorbitol was excluded

as these carbohydrates had no inhibitory effect on the generation of peroxides over time. Since  $\text{Fe}^{3+}$  reacts with xylenol orange a further technique was used to evaluate the proportion of  $\text{Fe}^{3+}$  freed by FeD and FeS.

5 The results from Fig. 9 show that, as expected, the positive control  $\text{FeCl}_3$  binds strongly with cysteine, as opposed to FeD and FeS. From the slope of the linear regressions for FeS ( $r^2=0.96$ ) and FeD ( $r^2=0.99$ ), it appears that 9% of FeS can bind with the thiol function

10 of cysteine, and only 2% in the case of FeD.

### Example III

#### **Protection by infusions sets of peroxidation of parenteral nutrition solutions**

15 The effect on TPN solutions of five sets of bags and tubing offering different levels of protection against light was evaluated (Fig. 10). The solution infused through transparent bag and tubing unprotected from light was used as control (C). In all other set-

20 tings TPN bags (Viaflex, Baxter, Mississauga, Ont, Canada) were protected from light with an opaque black plastic bag. The TPN solutions were run at 6 ml/h through 4 different types of tubing cut to a length of 2 m (inner volume of 10-12ml): (1) transparent tubing

25 (IVAC Corporation, San Diego, CA, USA); (2) orange tubing (MRT, Montreal, QC, Canada); (3) yellow tubing (Mixieva<sup>TM</sup> LPA, Miramed product line, Mirandola, Italy); (4) black tubing completely opaque (Miraflo<sup>TM</sup>, Miramed product line, Mirandola, Italy). The results obtained

30 in ambient light (16-32 fc:foot candel) were compared with those observed under phototherapy (65 fc). Two types of TPN solutions differing by their lipid content were used:

1. Fat-free TPN solution with the following composition (corresponding nutrient intake): total volume
- 35

- 150 ml (150 ml/kg/d); glucose 10% wt/vol (15 g/kg/d); amino acids 1.67% wt/vol (2.5 g/kg/d) (Primene<sup>TM</sup>, Baxter-Clintec, Mississauga, Ont, Canada); Na 20 mM (3 mmol/kg/d); K 13 mM (2 mmol/kg/d); Ca 6.6 mM (1 mmol/kg/d); monobasic phosphate 233 mg/L (35 mg/kg/d); SO<sub>4</sub>: 0.87 mM (0.13 mmol/kg/d); Mg 0.87 mM (0.13 mmol/kg/d); acetate 30 mM (4.496 mmol/kg/d); Cl 16.5 mM (2.5 mmol/kg/d); Se 13 µg/L; I 20µg/L; Cr 2.7µg/L; Mn 66.7µg/L; Cu 266µg/L; Zn 2 mg/L; multivitamins 1.6% vol/vol (2.5 ml/d) (MVI pediatric<sup>TM</sup>, Rhone-Poulenc-Rohrer, Montreal, Qc, Canada).
2. The same TPN solution containing a 8.5% vol/vol lipid emulsion (2.5g/kg/d) (20% Travemulsion, Baxter-Clintec, Mississauga, Ont, Canada) in a all-in-one preparation.

All TPN solutions used on a given day were initially prepared in one bag which was kept in darkness (<0.06 fc) after the addition of multivitamins. The initial solution was divided in darkness between the 5 experimental set ups shown in Fig. 10.

After 2 and 23 hours in ambient light, an aliquot of the solution was taken at the end of the tubing for the immediate determination of peroxide concentrations. Because a pilot study showed a faster generation of peroxides under those extreme conditions, a further determination was added at 1 hour with phototherapy and with the all-in-one TPN solution. After 3 and 24 h an aliquot was sampled for the immediate determination of thiol functions. All measurements were performed in darkness (light intensity <0.06 fc) and run in triplicate. The absorbance spectra of the different tubing and of 5'-phosphate flavin mononucleotide were determined on a spectrophotometer (Model DU-6, Beckman, Palo Alto, Ca, USA) between 235 and 900 nm.

Hydroperoxide concentrations were measured by the ferrous oxidation/xylene orange technique as described previously (Lavoie J.C. et al., *Pediatrics*, 1997, 99 (3):1-5; Jiang ZY et al., *Lipids*, 1991, 26:853-56). A 20  $\mu$ L sample was incubated during 30 minutes with 1ml of a solution containing  $H_2SO_4$  (22.5 mmol/L), xylene orange (90  $\mu$ mol/L),  $FeCl_2$  (225  $\mu$ mol/L), and BHT (3.6 mmol/L) in methanol. When required, lipids were separated by centrifugation (5500g, 3 min.). The absorbency of the solution or the supernatant was read 560 nm. Results were expressed as  $\mu$ mol/L equivalents of TBH (tert-butylhydroperoxide) ( $\mu$ mol/L equ TBH). Intra- and inter-assay coefficients of variation were 4 and 5% respectively.

The thiol functions related to the concentration of cysteine in Primene<sup>TM</sup> amino acid solution were measured as described previously (Lavoie JC et al., *J. Pediatr. Gastroenterol. Nutr.*, 1997, 25:307-311). A 50 $\mu$ L sample was added to 950  $\mu$ L TRIS buffer (pH 7.6) containing 0.6 mmol/L 5,5,-dithiobis(2-nitrobenzoic acid). The absorbency was read at 412 nm and compared to a standard curve made with known concentrations of cysteine (Ellman GL., *Arch. Biochem. Biophys.*, 1959, 82:70-77). Results were expressed as mmol/L equivalent of cysteine (mmol/L equ Cys).

All reagents were purchased from Sigma Chemical Co. (St Louis, MI, USA) or Aldrich Chemical Co. (Milwaukee, Wis, USA). Results were expressed as mean  $\pm$  SEM, n=3, and compared by Shcheffé's s-test. The level of significance was set at  $p < 0.05$ .

Fig. 11 presents the levels of peroxides in the fat-free TPN solution delivered with the different infusion sets. After 23 h, the TPN solution with the unprotected bag and transparent tubing exhibited the highest peroxides concentration. Even if the TPN bag

was protected from light, the peroxides concentration was > 50% higher with a transparent tubing than with the other tubing. Peroxides concentrations were in the same range in the settings with the yellow, orange or  
5 black tubing after 2 and 23 h. The concentration of peroxides increased significantly over time only with the settings with the transparent tubing. The level of thiol functions (Fig. 12), declined significantly by  
10 33% between 3 and 24 h in the setting with the black, yellow or orange tubing, and by 45% with the transparent tubing, and by 60% in the unprotected control.

When exposed to phototherapy (Fig. 13), the protective effect of the colored tubing was even more evident, since the peroxides concentrations were 2 to 4  
15 fold higher with the transparent tubing. The orange tubing had significantly less protective effects on peroxides concentrations after 1, 2 or 23 hours than the black one, with a 50% difference in peroxides concentrations after 24 h. The yellow tubing exhibited  
20 intermediate results after 24 h, with a 25% higher peroxide concentration than with the black setting. Phototherapy did not modify significantly the thiol functions in solutions infused through colored tubing. However, this type of light exposure accelerated  
25 significantly the disappearance of thiol functions in both solutions infused with a transparent tubing (at 3h:  $58 \pm 2\%$  with the protected bag;  $75 \pm 1\%$  with the unprotected bag). In the control setting only  $8 \pm 1\%$  of the initial thiol function ( $2.6 \text{ mmol/L equ Cys}$ ) remained  
30 after 24 h.

Fig. 14 presents the levels of peroxides in the TPN solution containing lipids delivered with the different infusion sets. Peroxides concentrations were not significantly different with the yellow or the  
35 black tubing. However, a rise (> 50%) in peroxides



concentration was noted with the orange tubing after 1 and 24 hours, and a 2 to 3.5 fold increase with the transparent tubing.

5 The spectra of absorption of the orange and the yellow tubing covers these wavelengths of absorption of MVI and riboflavin, however the absorbance is 2 fold higher with the yellow than with the orange tubing within the range of wavelengths of riboflavin absorption (Fig. 15).

10 The results show that the yellow tubing is as efficient as a completely opaque black tubing in preventing further peroxidation of TPN solutions in ambient light. As opposed to the black tubing, the yellow one is, from a clinical stand point, more appropriate  
15 to visually detect air bubbles, flocculation or precipitation during the infusion. This type of tubing associated with a light protected bag is a practical solution to diminish the peroxide loads received by parenterally fed patients. In view of the rapid rate  
20 of formation of these peroxides (Figs. 11 & 14) they can be generated during the preparation and storage of the TPN solution if the bag is not protected from light. This is all the more important if an all-in-one preparation is considered. Therefore, the type of protection  
25 bestowed by the yellow tubing could even be extended to TPN bags.

If no form of light protection is available, it appears from the results presented in Fig. 14 that one should attempt to decrease the duration of exposure of  
30 a complete TPN admixture to light. Therefore, to minimize peroxide generation the lipid emulsion should be "piggy-backed" on to the transparent tubing, as close as possible to the infusion site on the patient.

The multivitamin preparation is the major contributor  
35 to peroxide generation in TPN solutions.

Darkness prevents the generation of peroxides in the multivitamin preparation. And 5'-phosphate flavin mononucleotide is a photoexcitable component of the multivitamin preparation which catalyzes this generation of peroxides in the presence of electron donors in TPN solutions.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A liquid multivitamin preparation comprising riboflavin and vitamin C for enteral or parenteral administration and a reduced amount of peroxide, wherein said riboflavin is not in contact with said vitamin C.
2. A liquid multivitamin preparation for parenteral or enteral administration comprising reduced amounts of peroxide, wherein said multivitamin preparation is solubilized in the presence of iron bound to a polyose molecule.
3. A multivitamin preparation according to claim 2, wherein said iron bound polyose molecule is selected from the group consisting of Iron Saccharide, Iron Dextran and Iron Sorbitol.
4. A multivitamin preparation according to claim 2, wherein the iron bound polyose molecule is Iron Dextran.
5. A liquid multivitamin preparation comprising riboflavin and vitamin C for enteral or parenteral administration and a reduced amount of peroxide, wherein said multivitamin preparation is in two phases until the time of enteral or parenteral administration, a riboflavin phase separated from a vitamin C phase up to a molar ratio of about 1/10,000 between concentrations of riboflavin and vitamin C.

6. A method of administering a liquid multivitamin preparation comprising riboflavin and vitamin C with reduced amounts of peroxide, wherein said method comprises the step of protecting the preparation from light.

7. The method of claim 6, wherein said method comprises the step of protecting the preparation from light having a wavelength of about 200nm to about 800nm.

8. The method according to claim 7, wherein said light has a wavelength of about 300nm to about 600nm

9. The method according to claim 7, wherein said light has a wavelength of about 325nm to about 550nm.

10. The method of claim 6, wherein said step of protecting the preparation from light is effected by means of a substantially opaque dispensing means for administering the multivitamin preparation.

11. The method of claim 6, wherein said step of protecting the preparation from light is effected by means of a colored dispensing means for administering the multivitamin preparation.

12. The method according to claim 11, wherein the dispensing means is colored by at least one color or a combination thereof selected from the group consisting of black, gray, brown, orange, yellow, green, blue, and red.

13. A method according to claim 12, wherein the color is selected from the group consisting of black, brown, orange and yellow.

14. A colored container or a substantially opaque container for administering a liquid multivitamin preparation in order to protect the multivitamin preparation from light.

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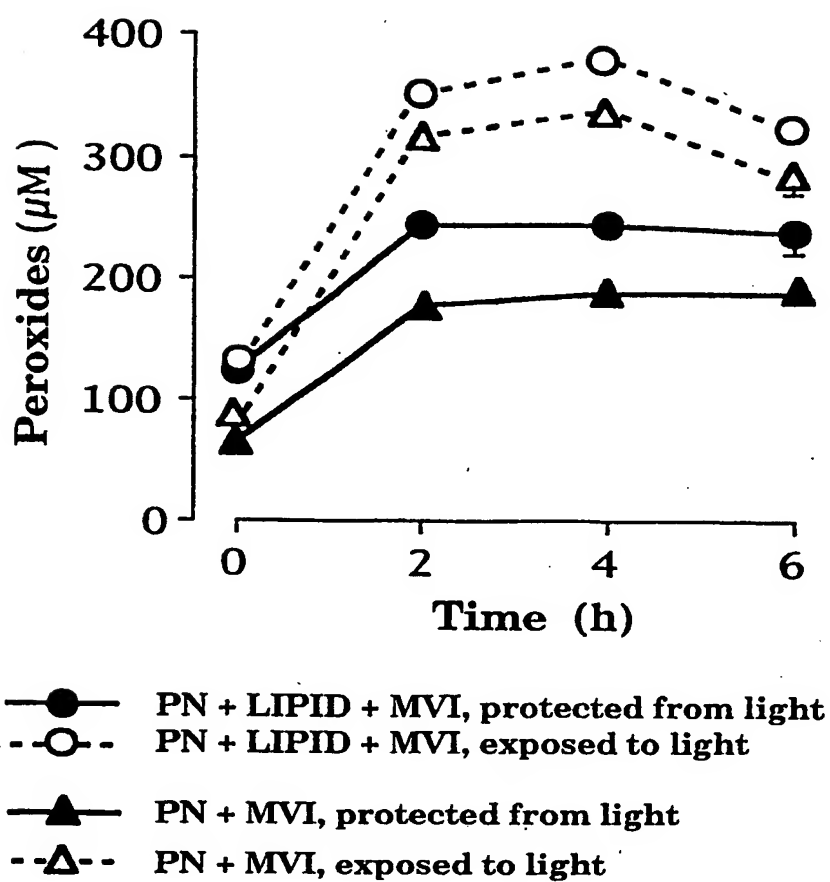


Fig. 1

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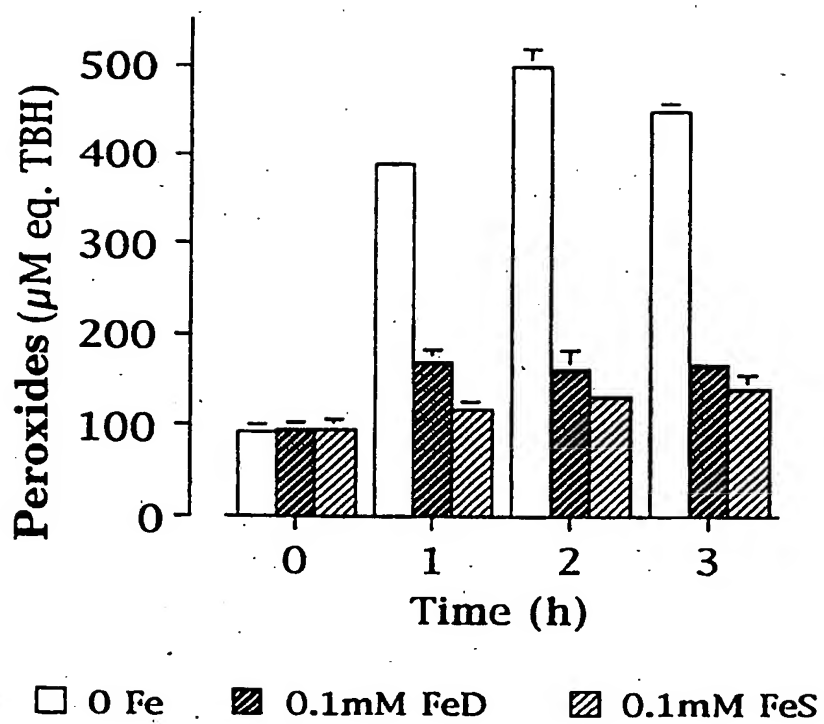


Fig. 2

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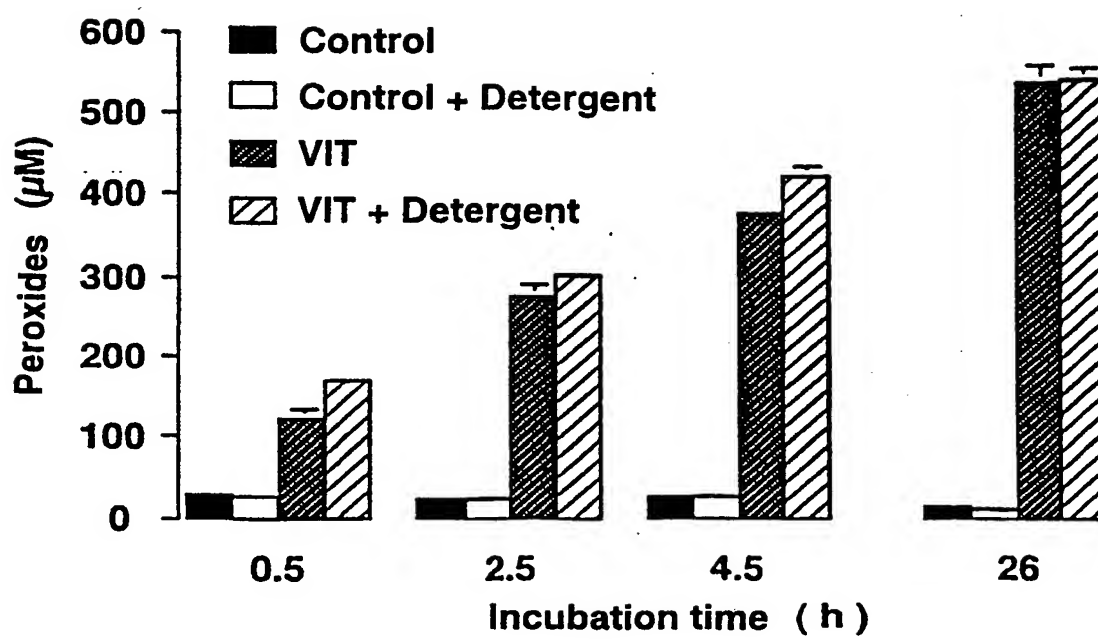


Fig. 3



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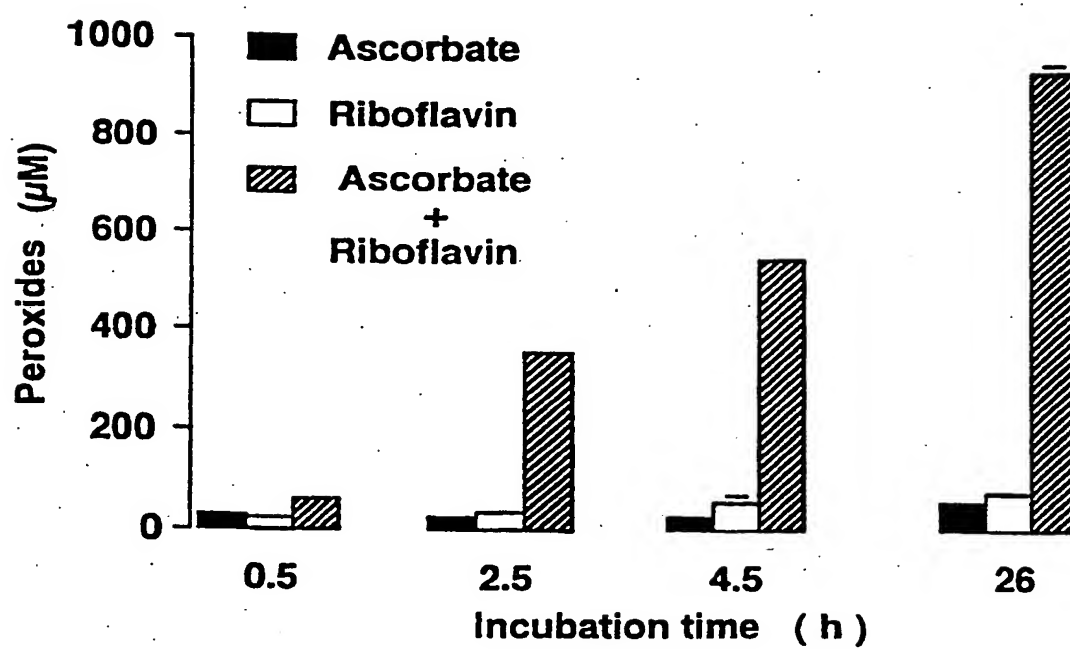


Fig. 4

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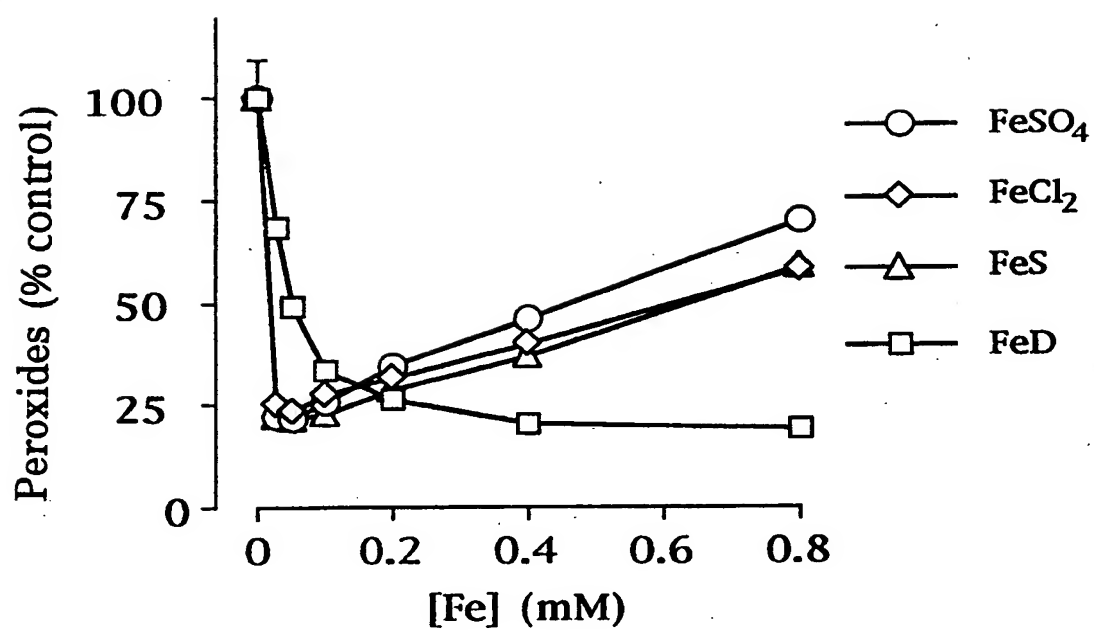
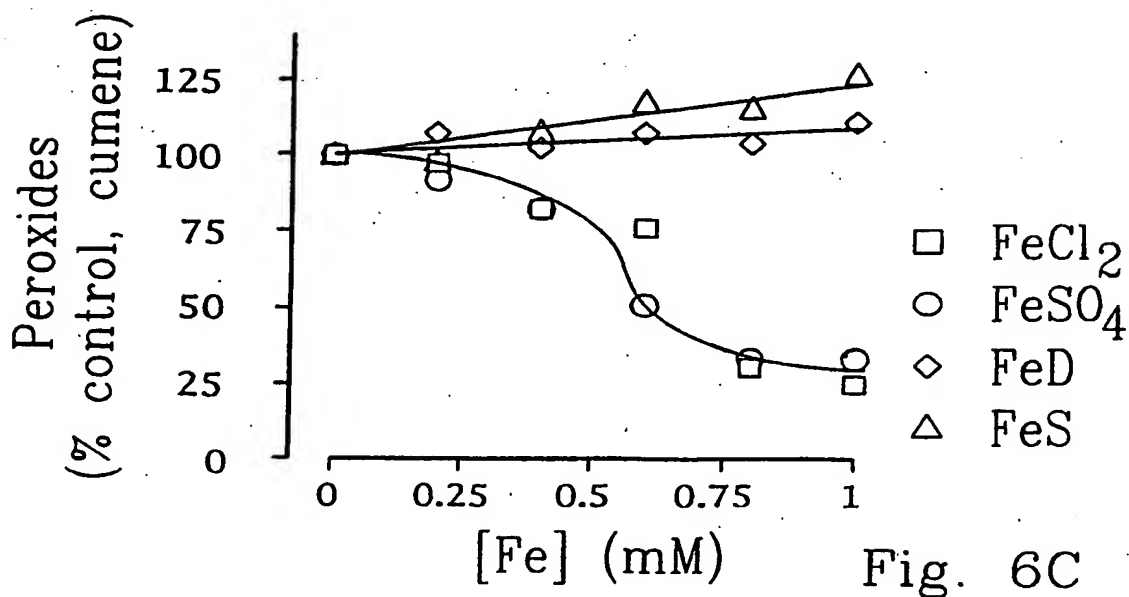
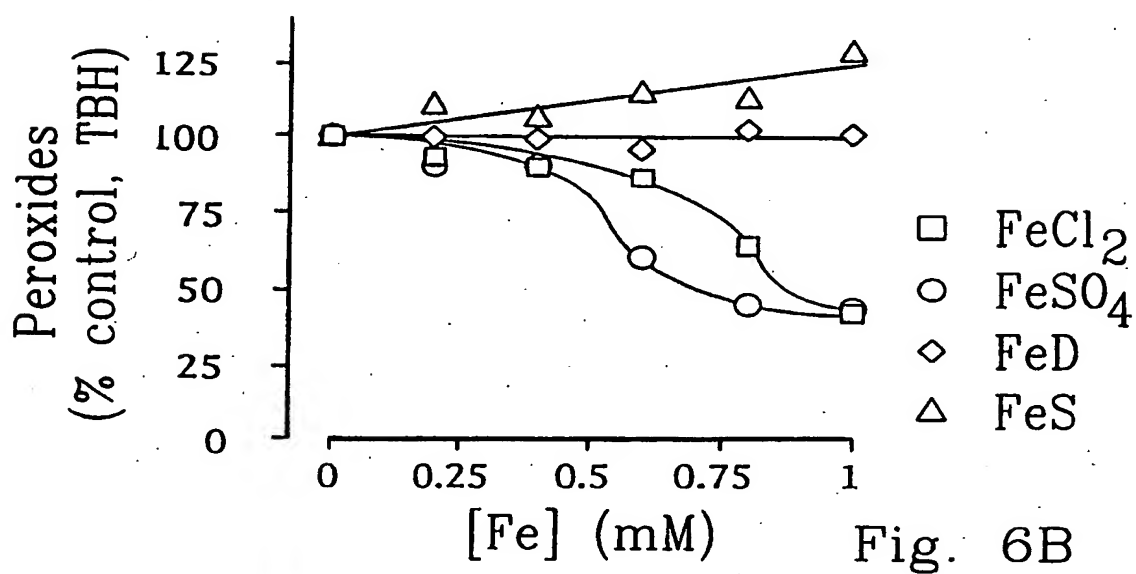
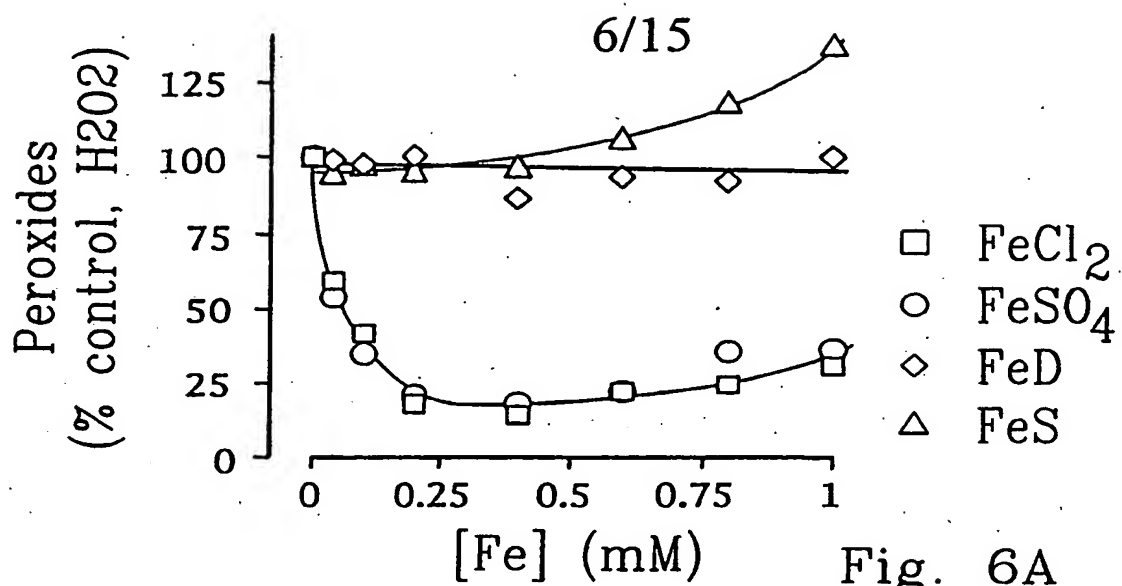


Fig. 5



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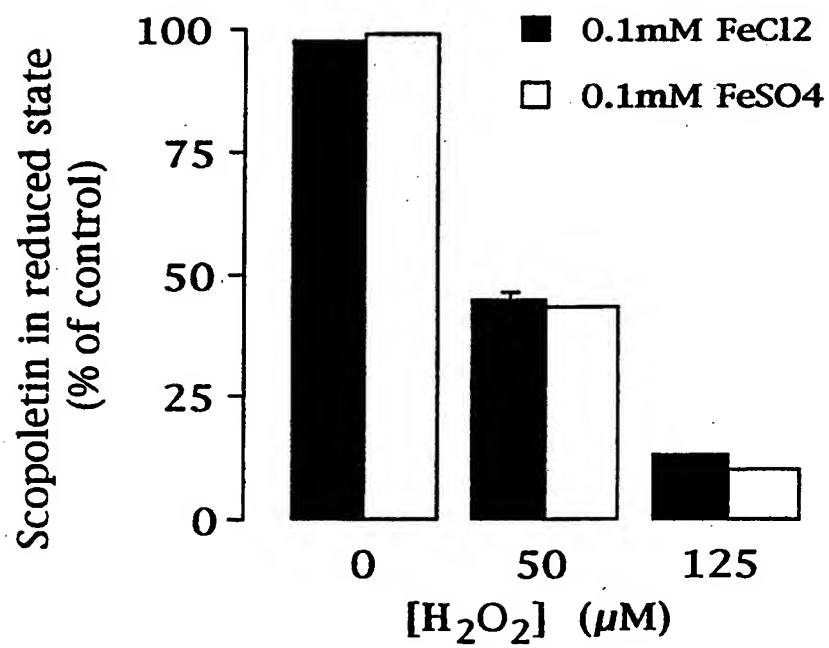


Fig. 7

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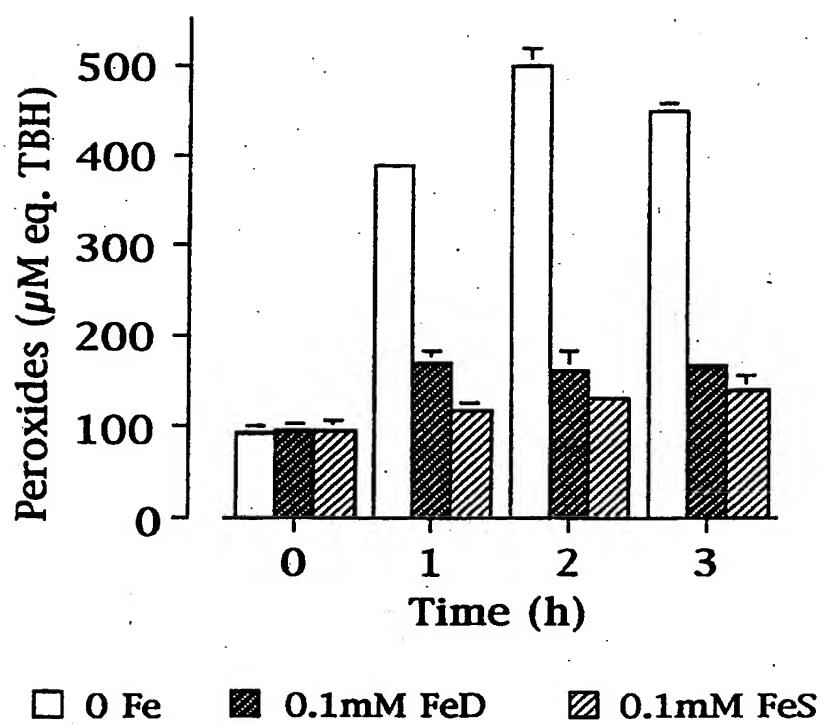


Fig. 8

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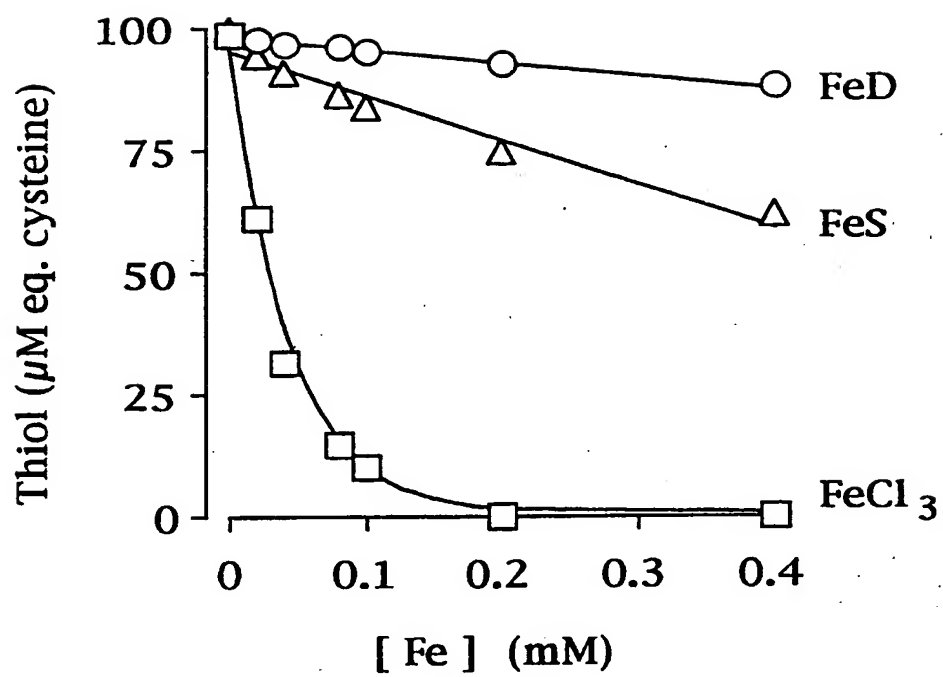


Fig. 9

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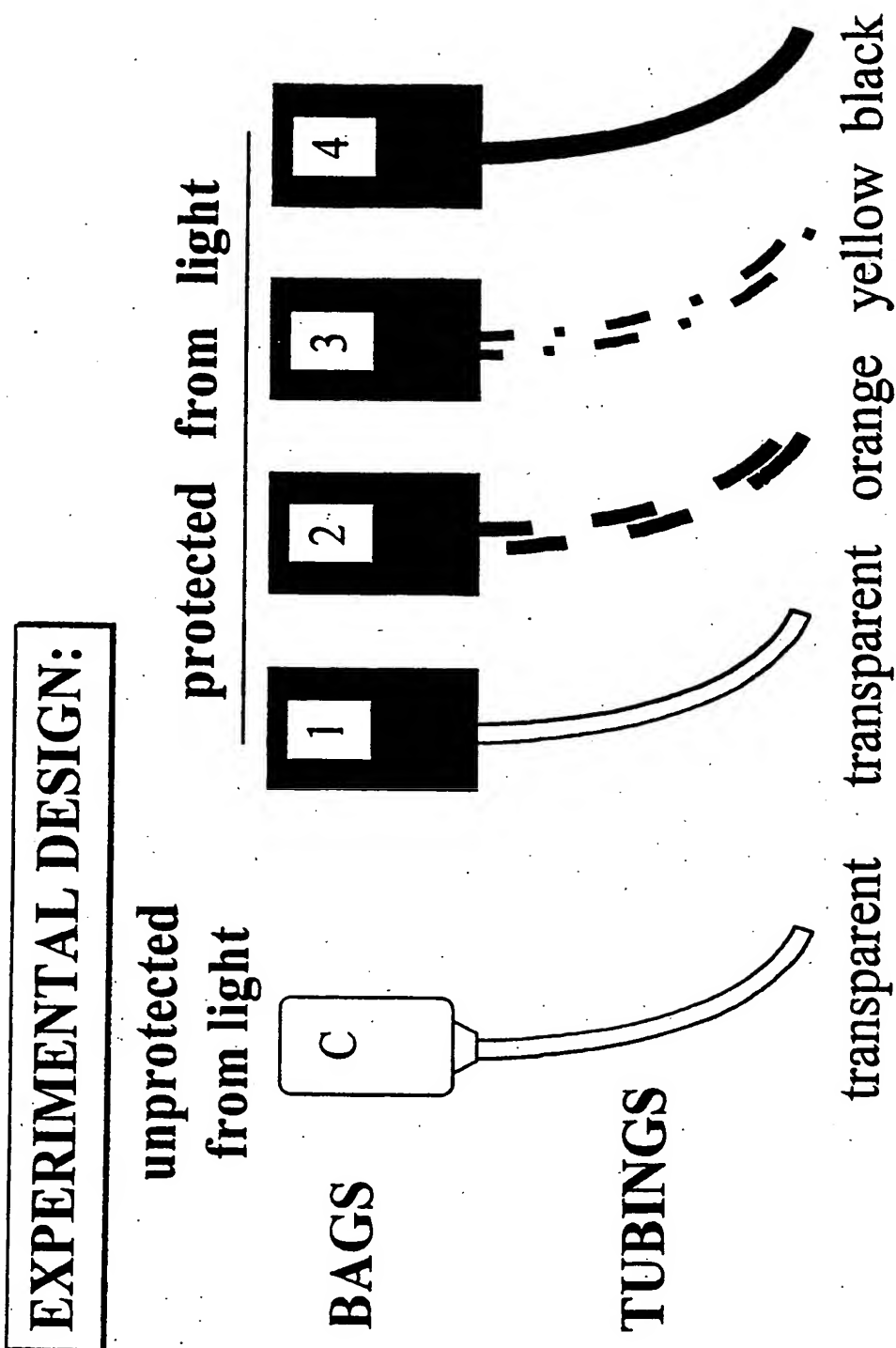


Fig. 10

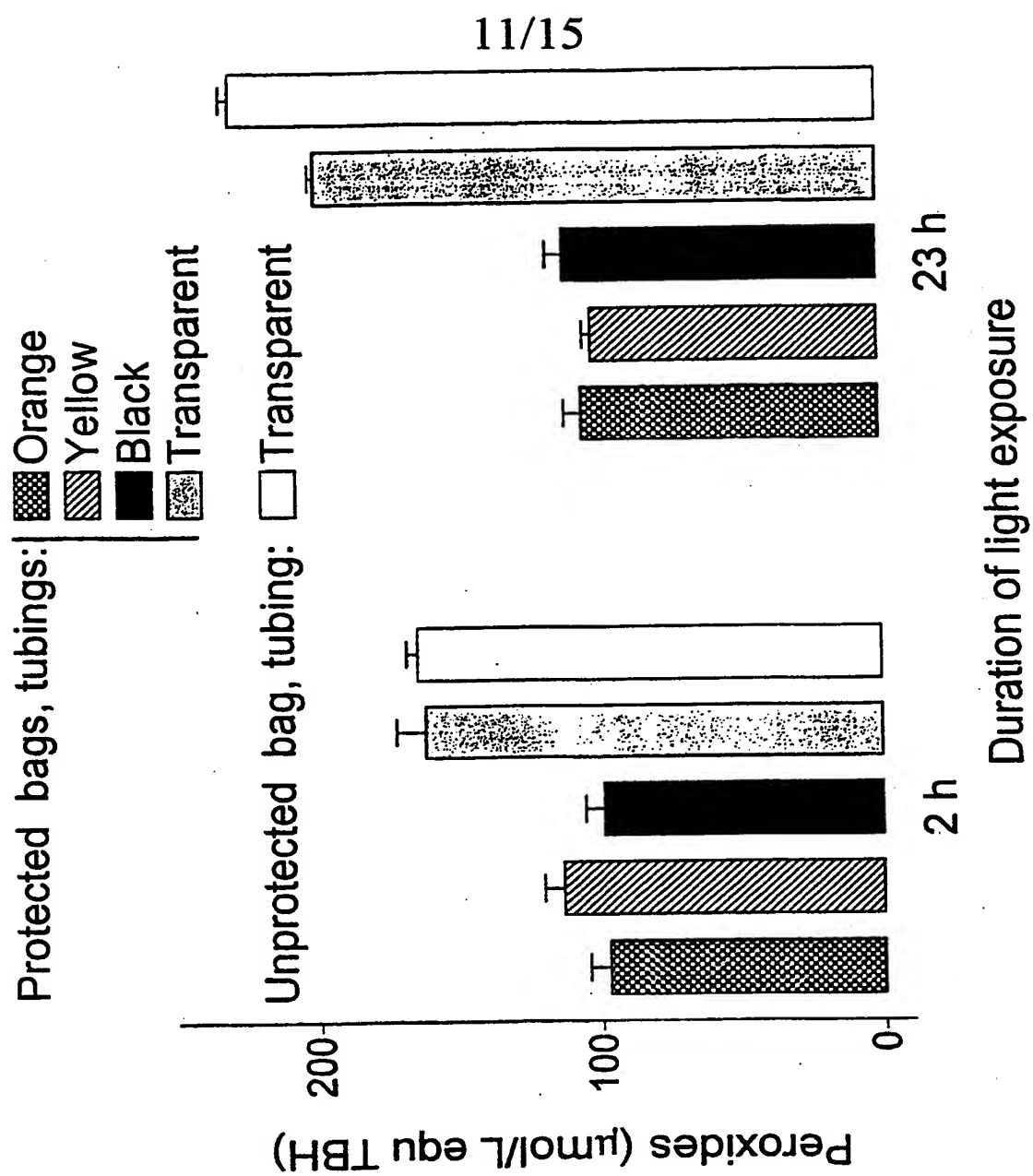


Fig. 11



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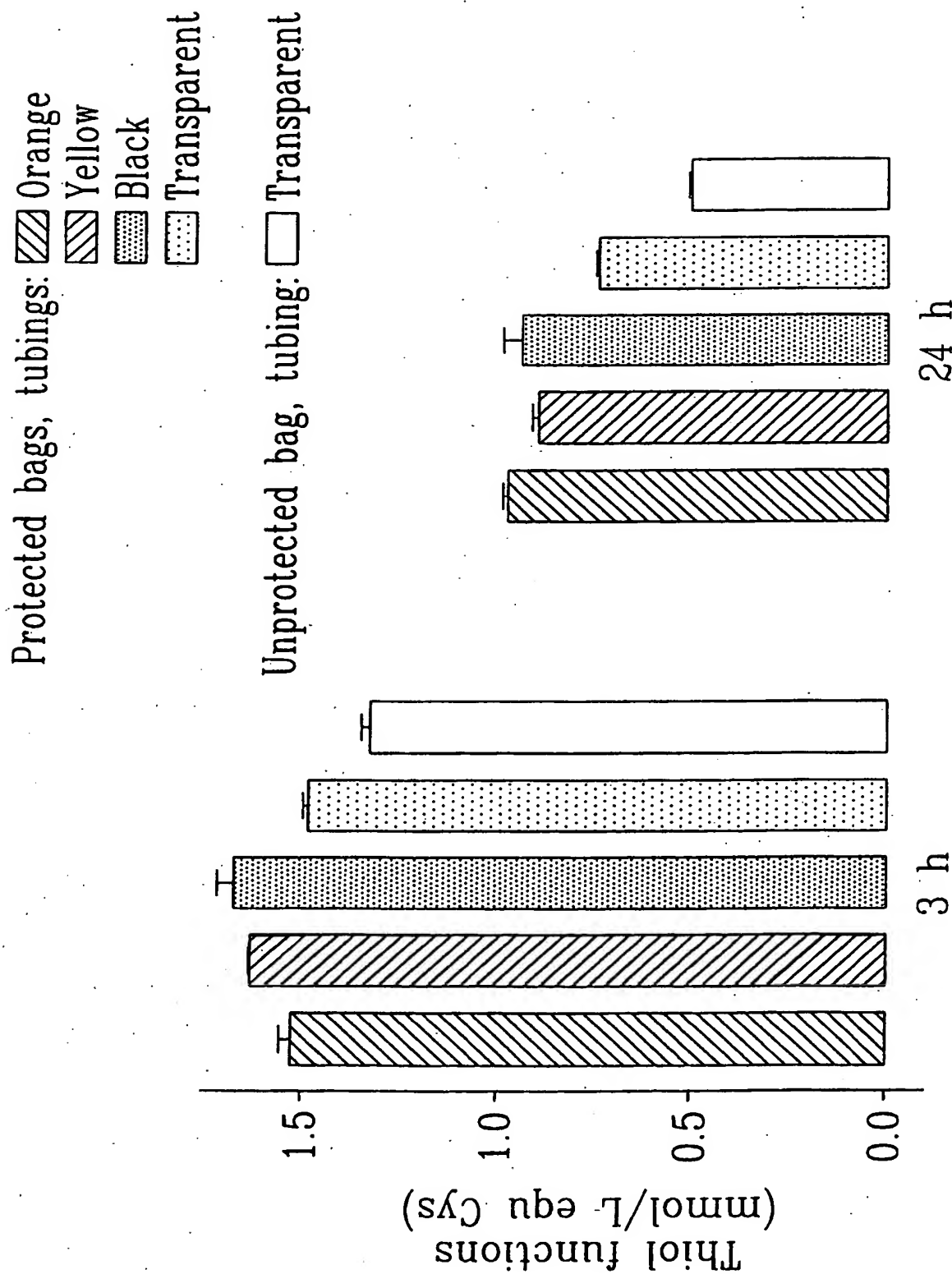
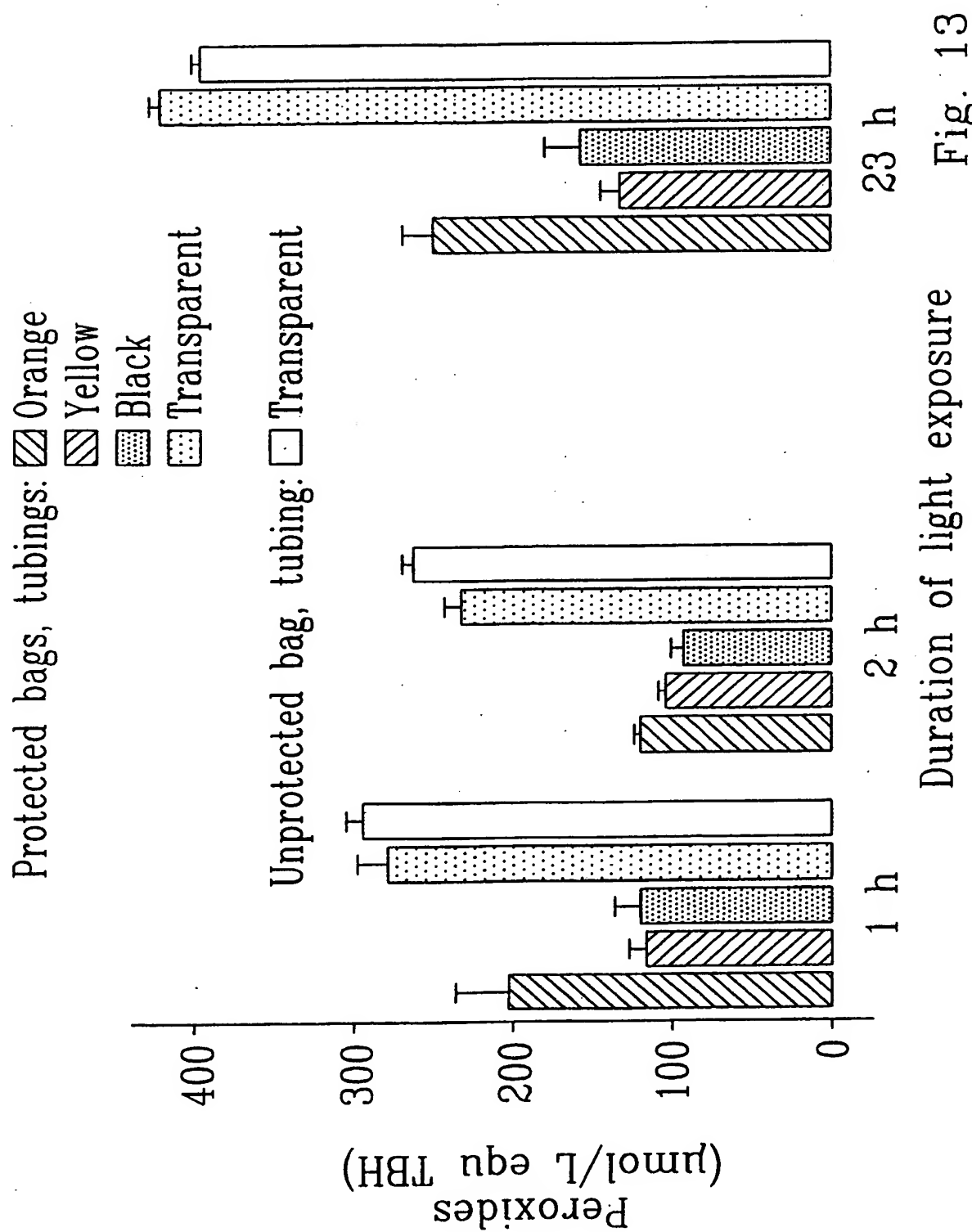


Fig. 12

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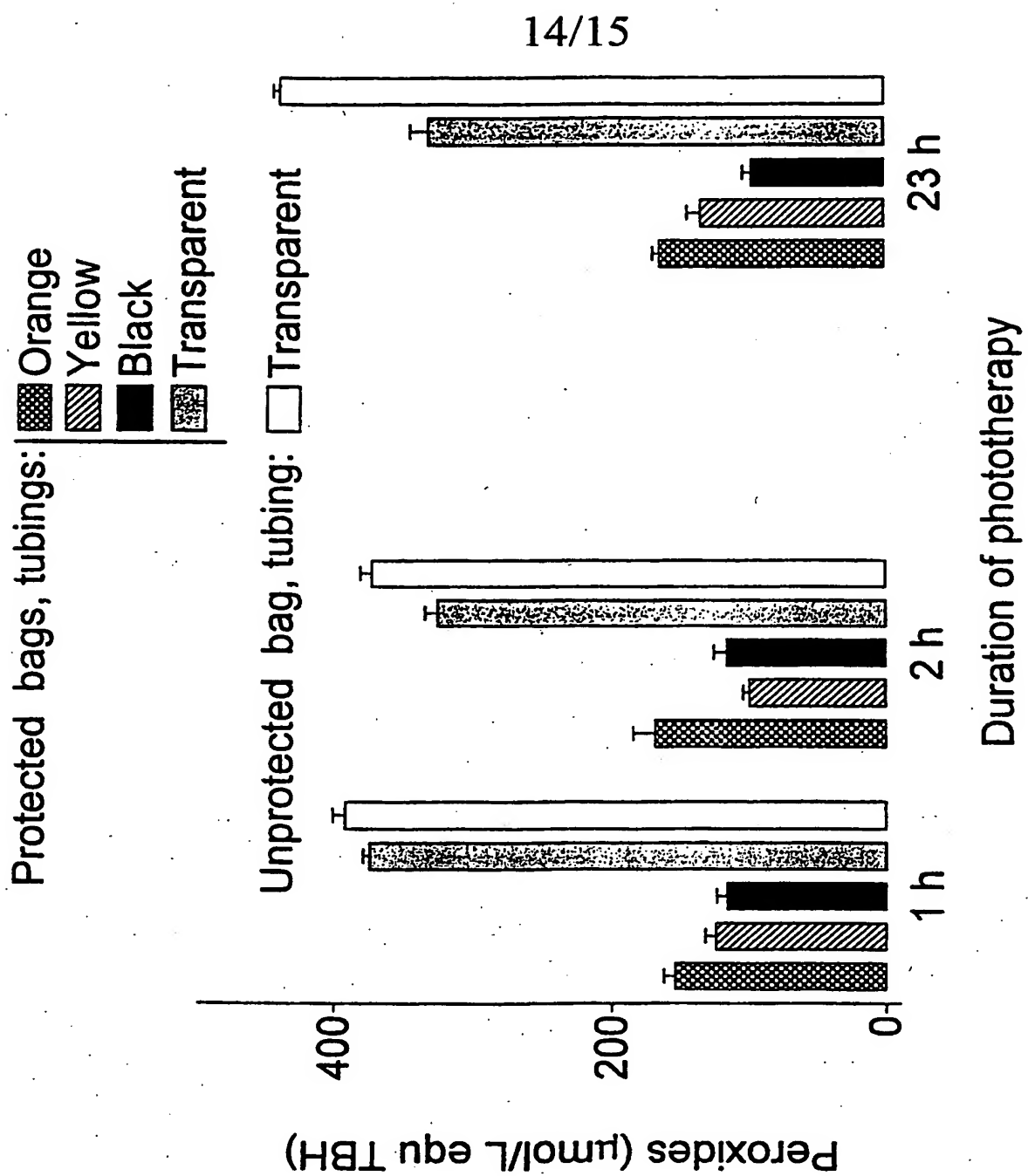


Fig. 14

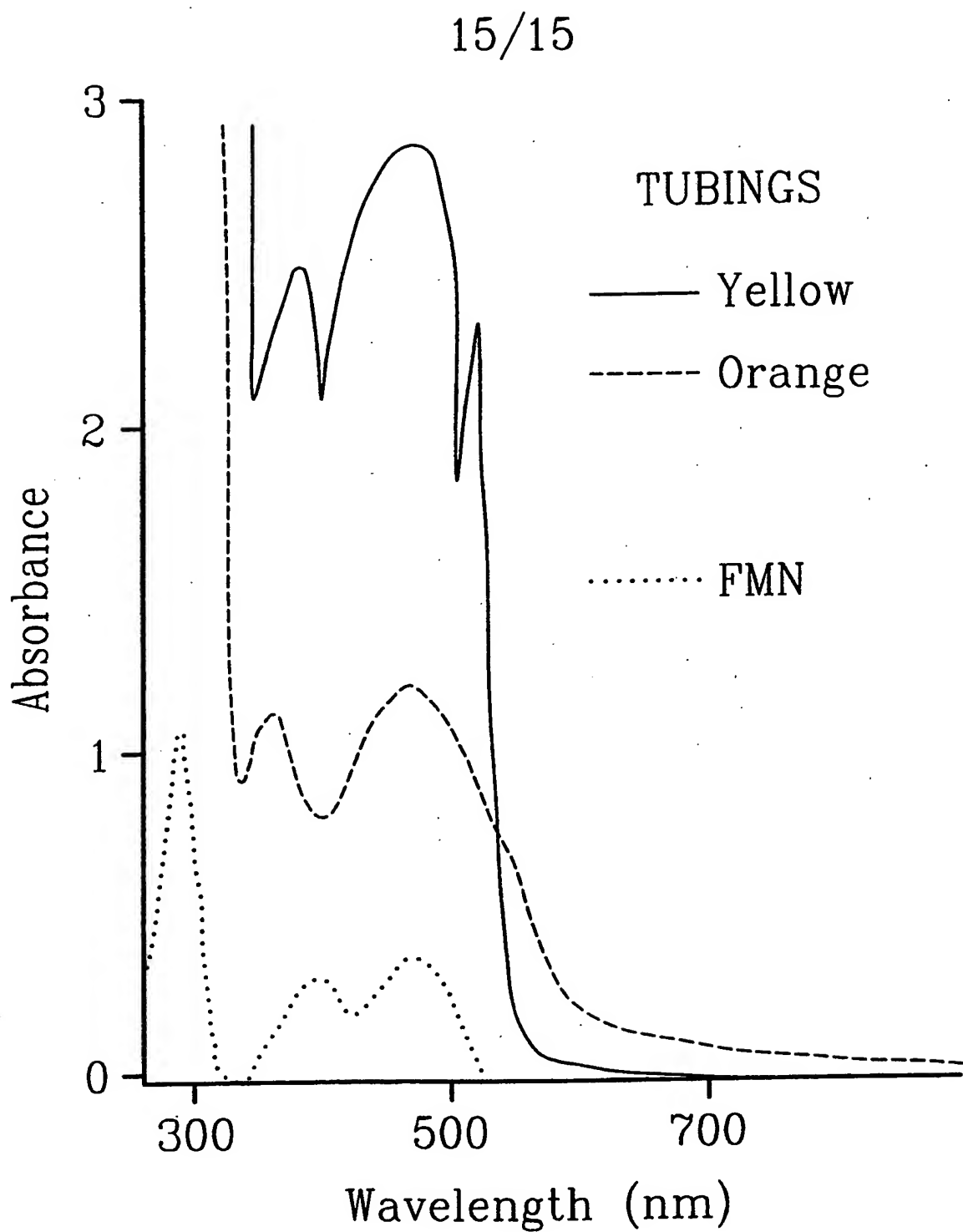


Fig. 15

# INTERNATIONAL SEARCH REPORT

Int .tional Application No  
PCT/CA 98/00342

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A23L1/302 A23L1/304 A61K31/375 A61K31/525 A61K31/295

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data-base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 362 719 A (FARBWERKE HOECHST AKTIENGESELLSCHAFT) 7 August 1974 see page 2, line 67 - line 79; claims 1-3	2-4
X	J.O.BOSSET ET AL.: "Einfluss der Lichtdurchlässigkeit der Verpackung auf die Haltbarkeit von Milch und Milchprodukten - eine Übersicht" MITTEILUNGEN AUS DEM GEBIETE DER LEBENSMITTELUNTERSUCHUNG UND HYGIENE, vol. 84, no. 2, - 1993 pages 185-231, XP002074665	1,5-14
Y	see page 192 - page 194; tables 7,8 -/-	1-14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

17 August 1998

Date of mailing of the international search report

01/09/1998

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAZUKO SHIMADA ET AL.: "Iron-Binding Property and Antioxidative Activity of Xanthan on the Autooxidation of Soybean Oil in Emulsion" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 42, no. 8, - 1994 pages 1607-1611, XP000465844	2-4
Y	see summary	1-14
X	--- KAZUKO SHIMADA: "Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 40, no. 6, - 1992 pages 945-948, XP002074666 see page 946, right-hand column	2-4
X	--- ÜLFET SANSAL & GÜLER SOMER: "The kinetics of photosensitized decomposition of ascorbic acid and the determination of hydrogen peroxide as a reaction product" FOOD CHEMISTRY, vol. 59, no. 1, - 1997 pages 81-86, XP002074667	1,5-14
Y	see summary	1-14
X	--- FERHUNDE SAHBAZ & GÜLER SOMER: "Photosensitized decomposition of ascorbic acid in the presence of riboflavin" FOOD CHEMISTRY, vol. 46, no. 2, - 1993 pages 177-182, XP002074668 see summary	1,5-14

# INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No

PCT/CA 98/00342

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